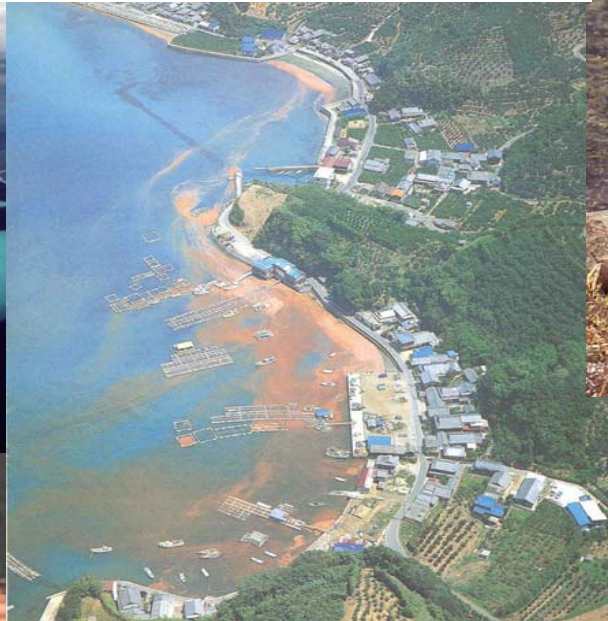


# Chemical Ecology of Toxic Algae

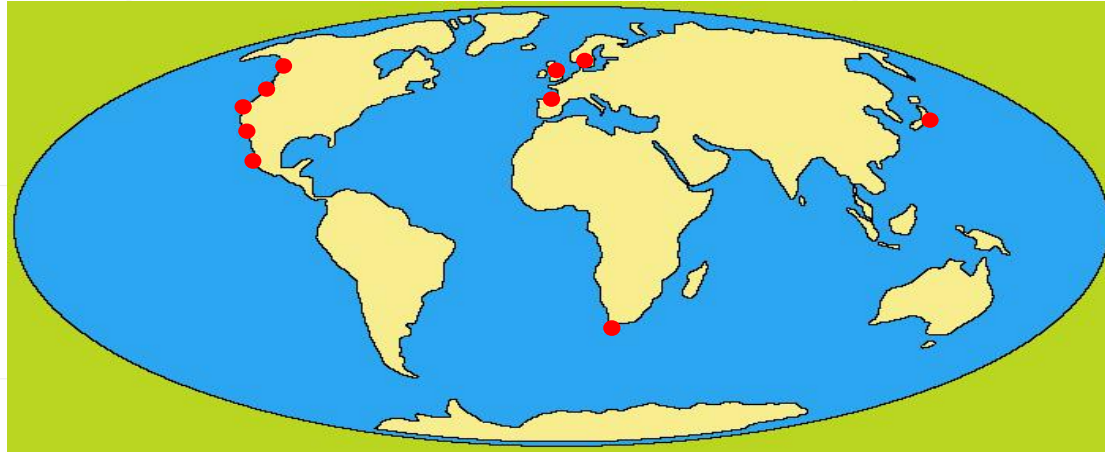
Bernd Krock, Urban Tillmann, Uwe John, Sára Beszteri,  
Chishimba M. Kantu, Allan D. Cembella

# Toxic Algal Blooms

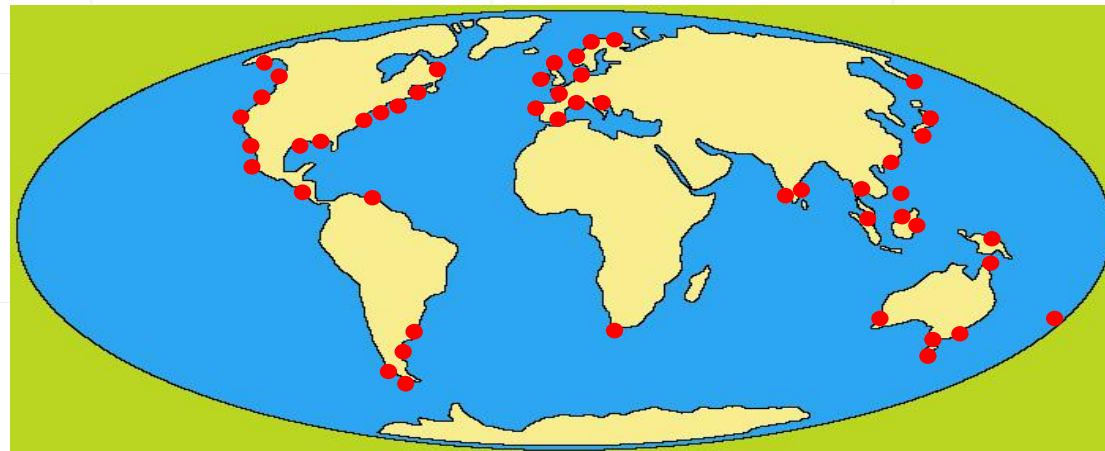


# Distribution of Paralytic Shellfish Poisoning events

**1970**



**2005**





# Lytic Effect of *Alexandrium*

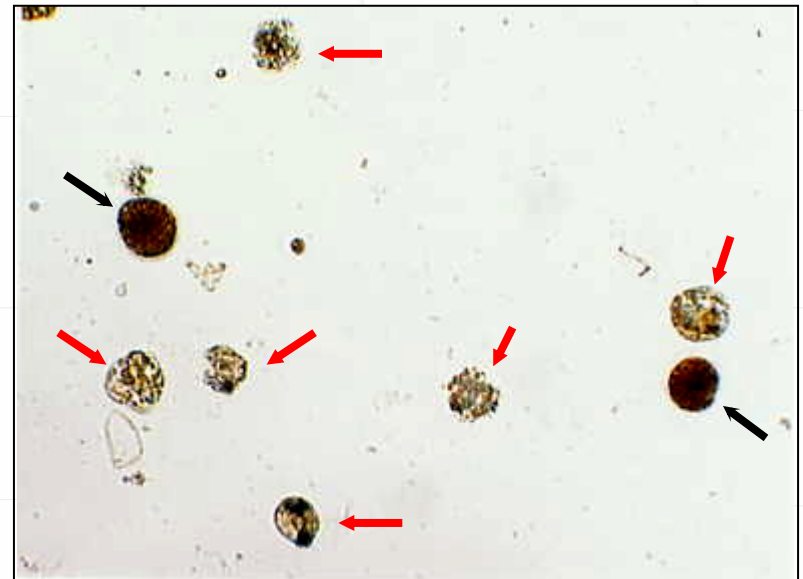


*Oxyrrhis marina*



*Alexandrium tamarense*

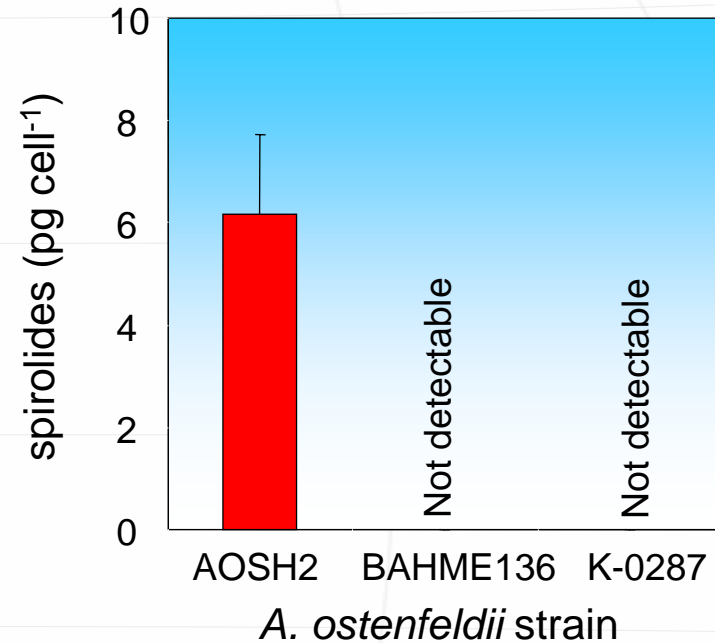
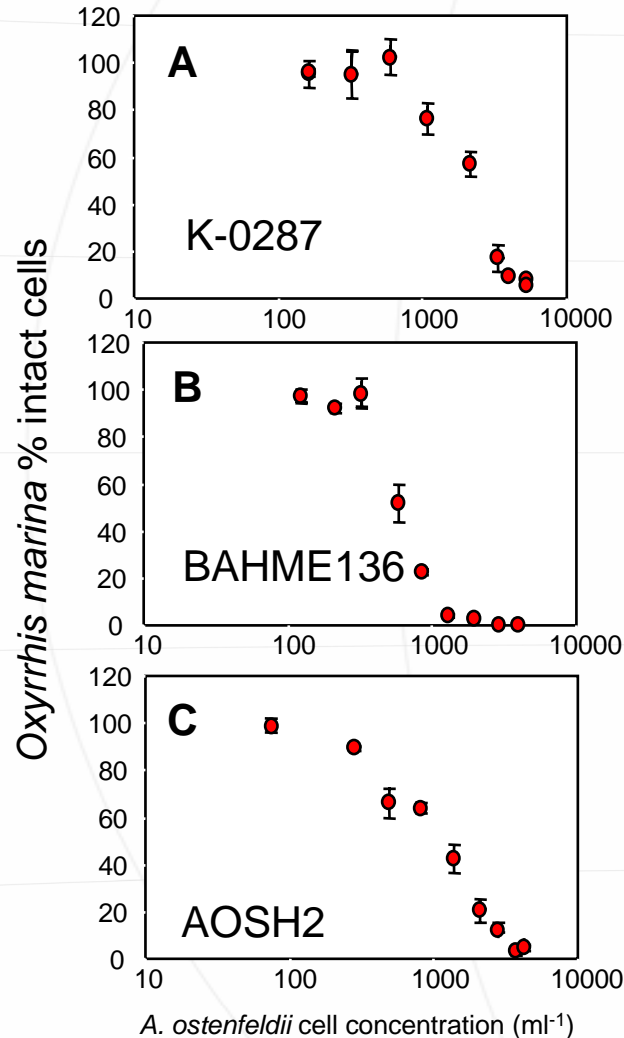
Photos: U. Tillmann



Lytic Effect of *Alexandrium* shown with *Oxyrrhis marina*.

Black arrows: *Alexandrium*  
Red arrows: Remnants of *Oxyrrhis*

# Lytic Effect of *A. ostenfeldii*



**Allelochemical potency is not related to spirolide production**

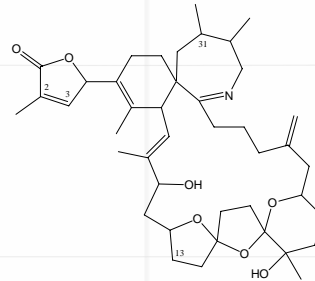
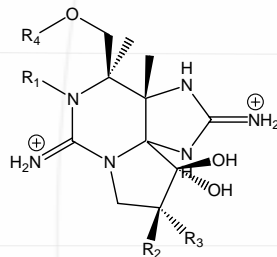
# Organism    Chemical Interaction    Ecological Function

*Alexandrium tamarense*

PSP-Toxins

Spirolides

*Alexandrium minutum*



?

*Alexandrium ostenfeldii*

*Alexandrium tamarense*

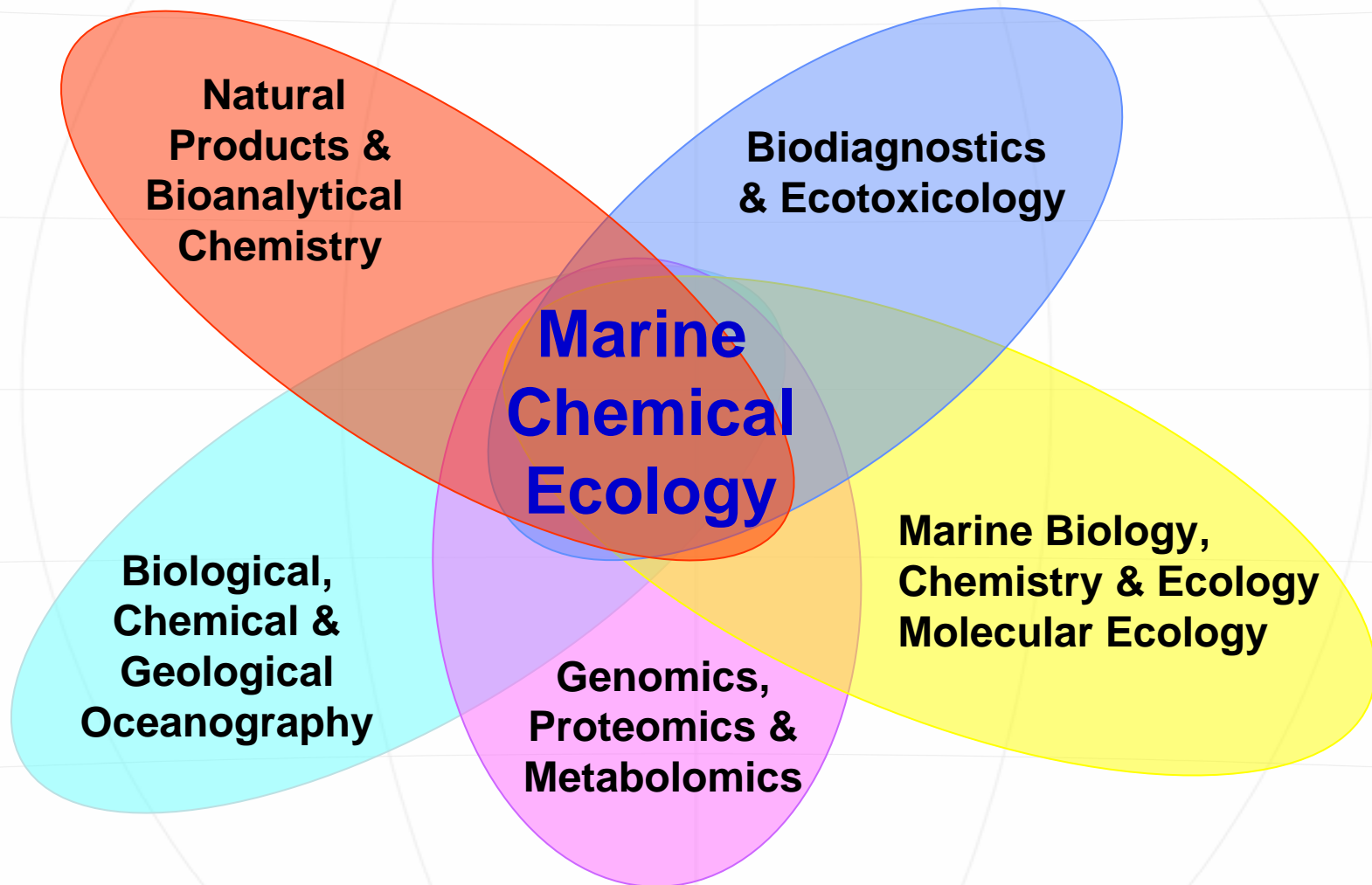
?

Defense against Predators,

*Prymnesium parvum*

Elimination of Competitors

# Towards Inter-disciplinary Science



# Strategies to answer these questions:

**Can toxic strains be detected genetically?**

=> Genomic characterization (microsatellites, AFLP, rDNA sequence analysis)

**Which genes are responsible for growth and toxicity?**

=> Gene expression analysis (EST, Data bases, microarrays)

**What toxins are present?**

=> Bioanalytics (LC-FD, LC-MS/MS)

**What are the allelochemicals?**

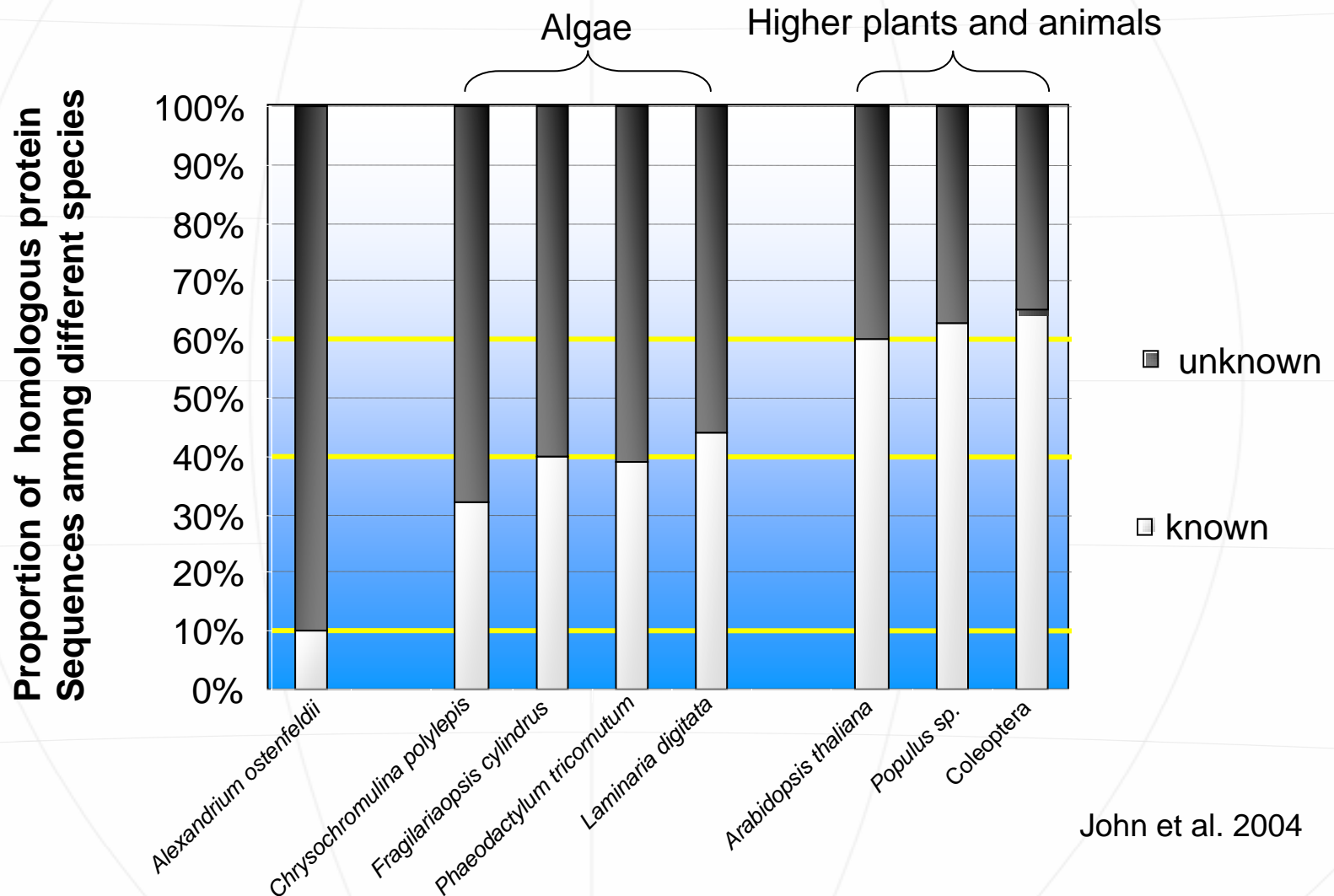
=> Chemical experiments & bioassays

**What effects do toxins have?**

=> Toxicological assays



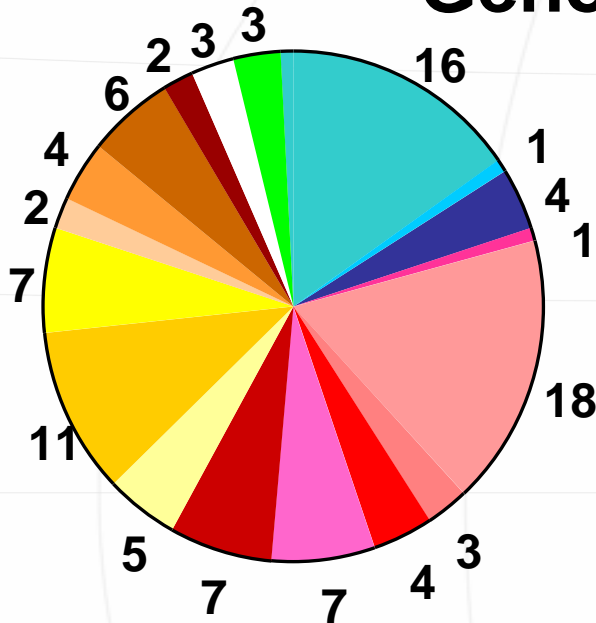
# Genomic Characterization



John et al. 2004

# Genomic Characterization

## PKS EST Analysis



Cell structure

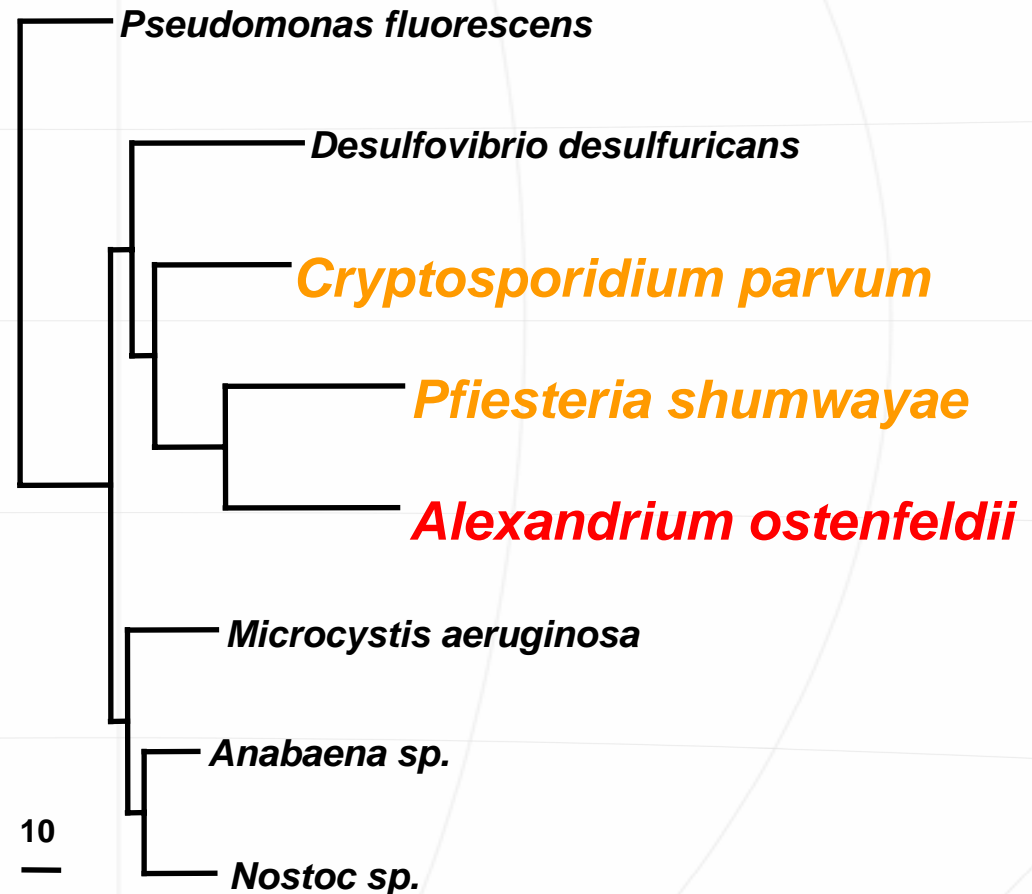
Information storing and processing

Cellular processes

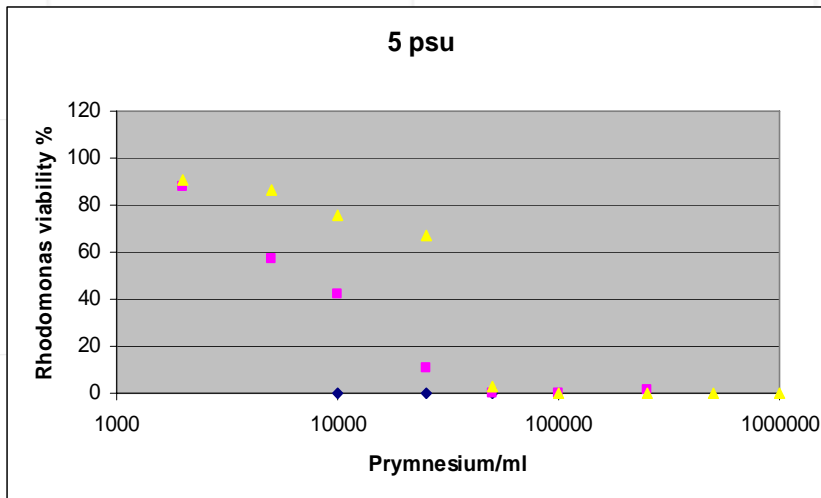
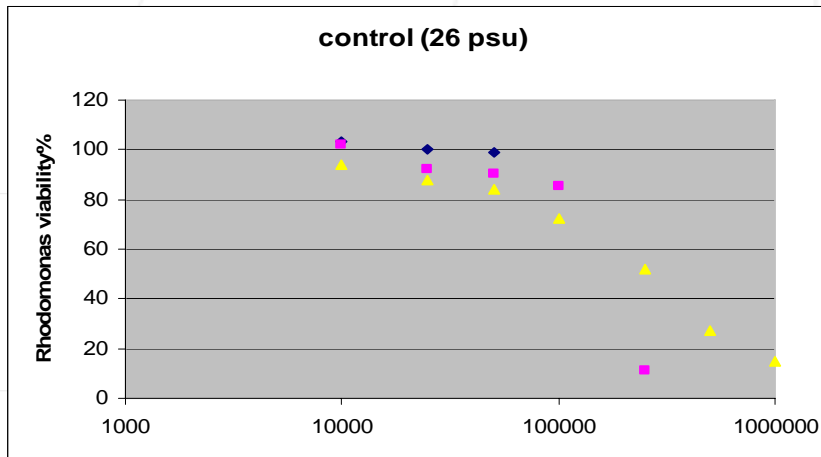
Metabolism

General function (prediction only)

Stress, defence and toxicity



# Gene Expression Analysis

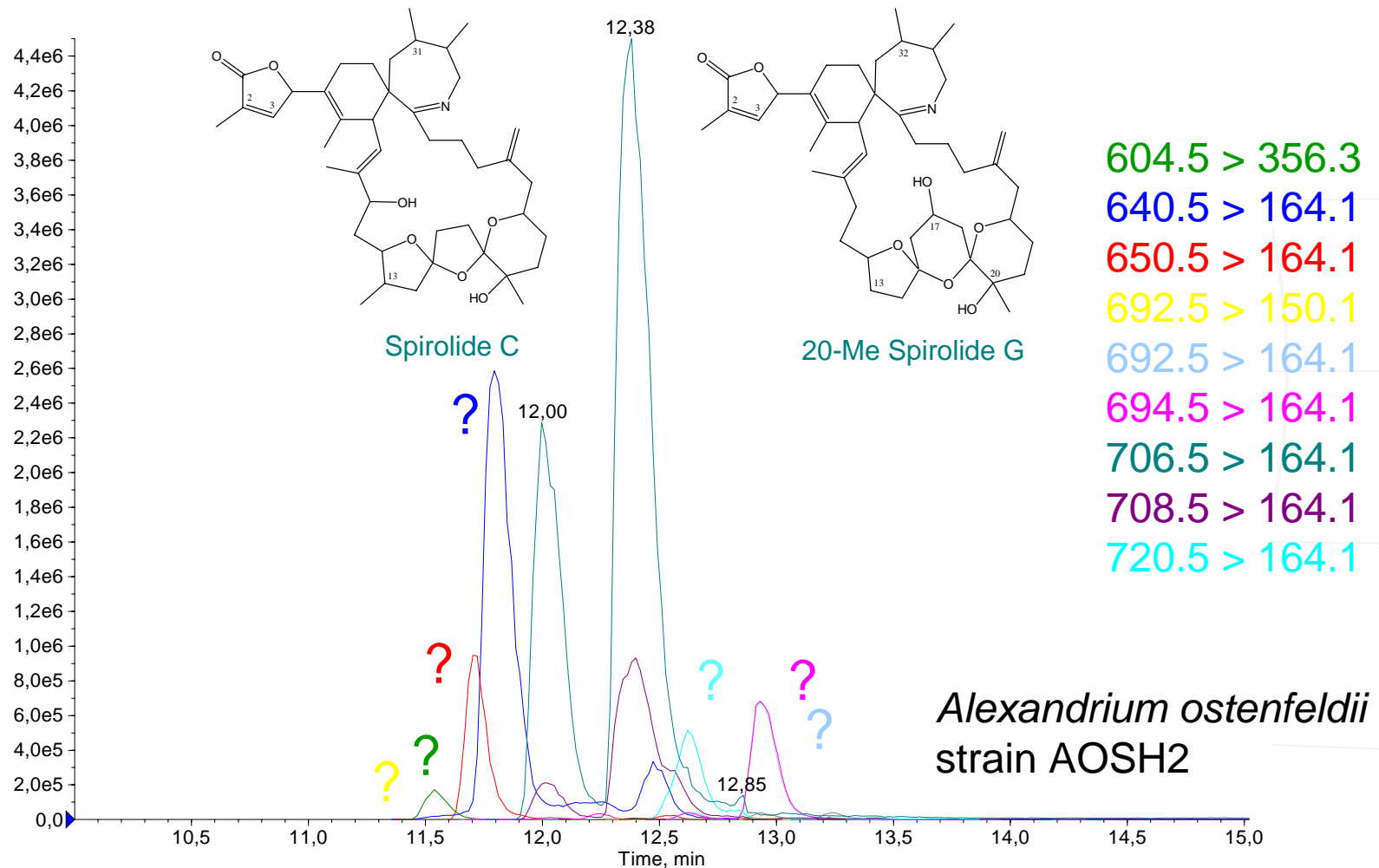


- Early exponential growth phase
- Late exponential growth phase
- Stationary phase

Toxicity of *Prymnesium parvum* is high at low salinity

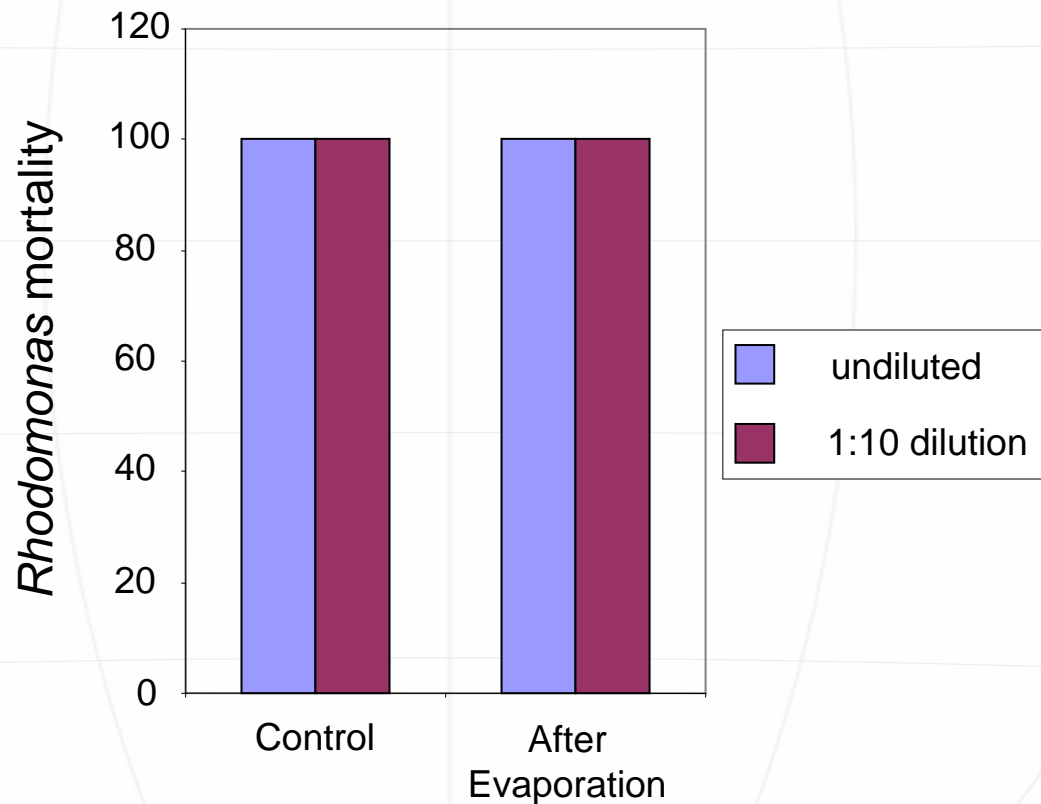
=> ESTs, Microarrays

# Bioanalytics



# Chemical Experiments – Evaporation

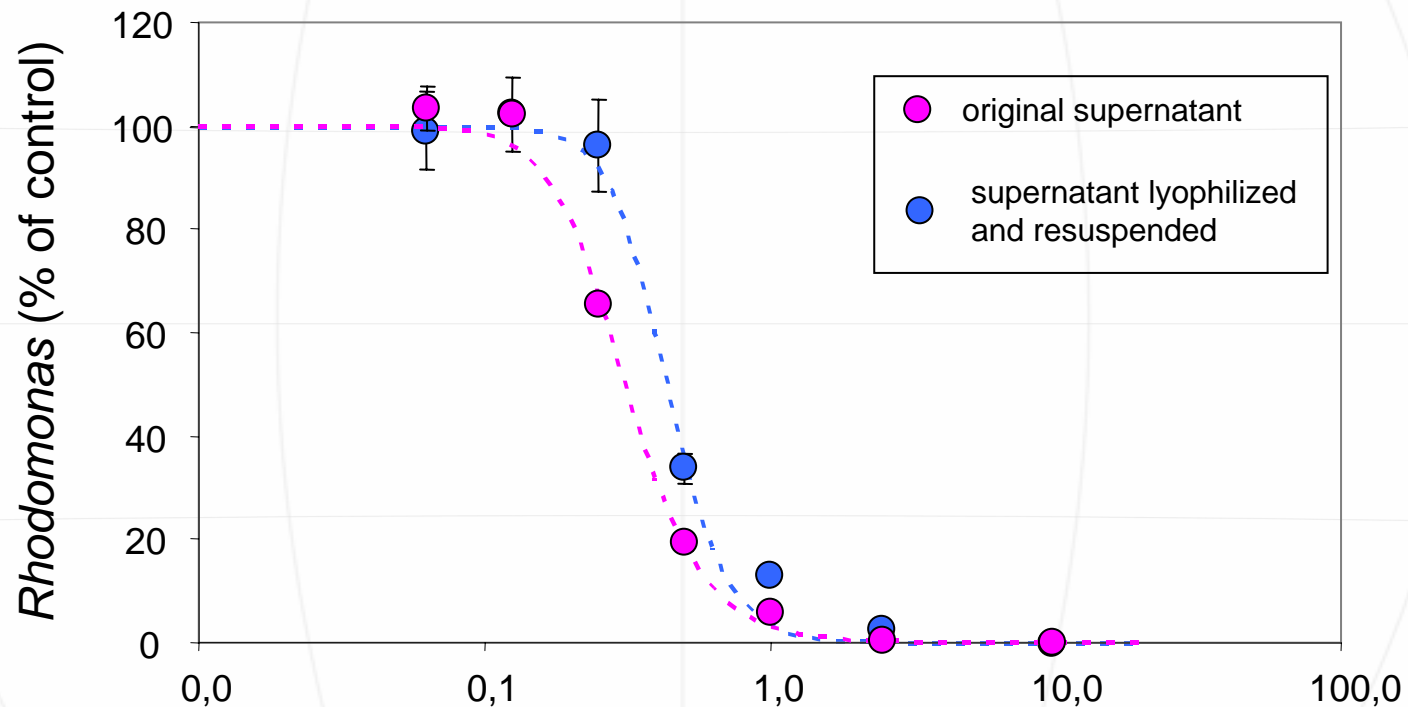
*Alexandrium tamarense* supernatant – Lytic Effect on *Rhodomonas*





# Chemical Experiments – Lyophilization

*Alexandrium tamarense* supernatant – Lytic Effect on *Rhodomonas*



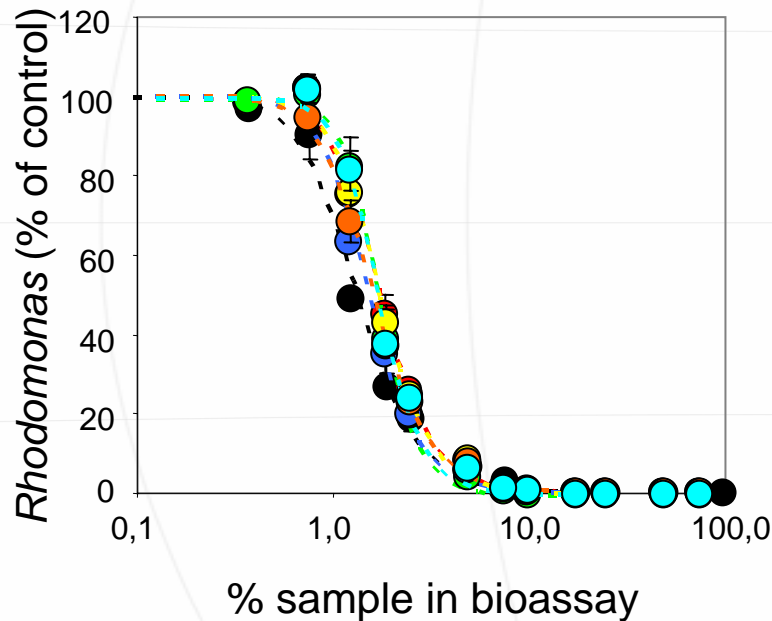
EC<sub>50</sub> original: 0.31 %    % sample in bioassay

EC<sub>50</sub> „instant“: 0.44 %

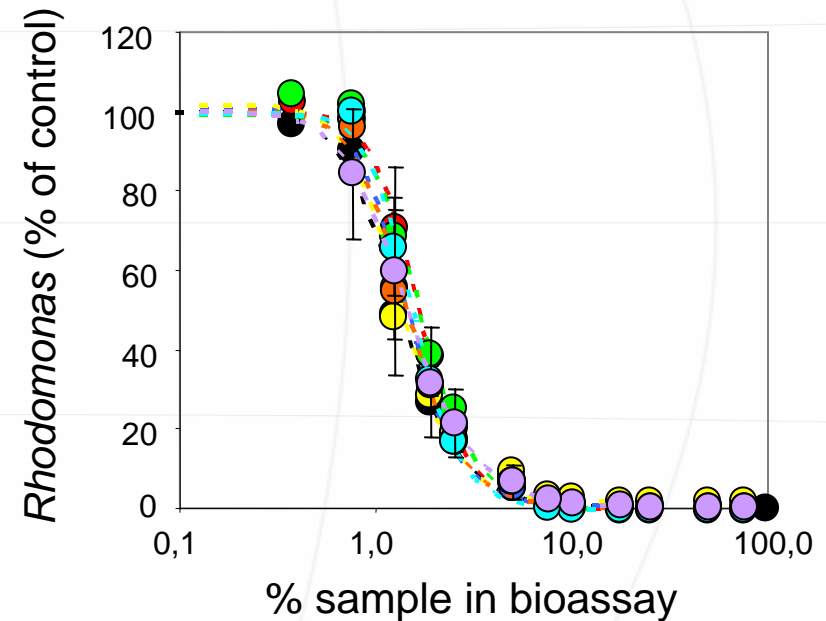
# Chemical Experiments – Stability

*Alexandrium tamarense* supernatant – Lytic Effect on *Rhodomonas*

15°C; light (150  $\mu\text{E m}^{-2} \text{s}^{-1}$ )



15°C; dark



● t = 0

● t = 4d

● t = 12d

● t = 49d

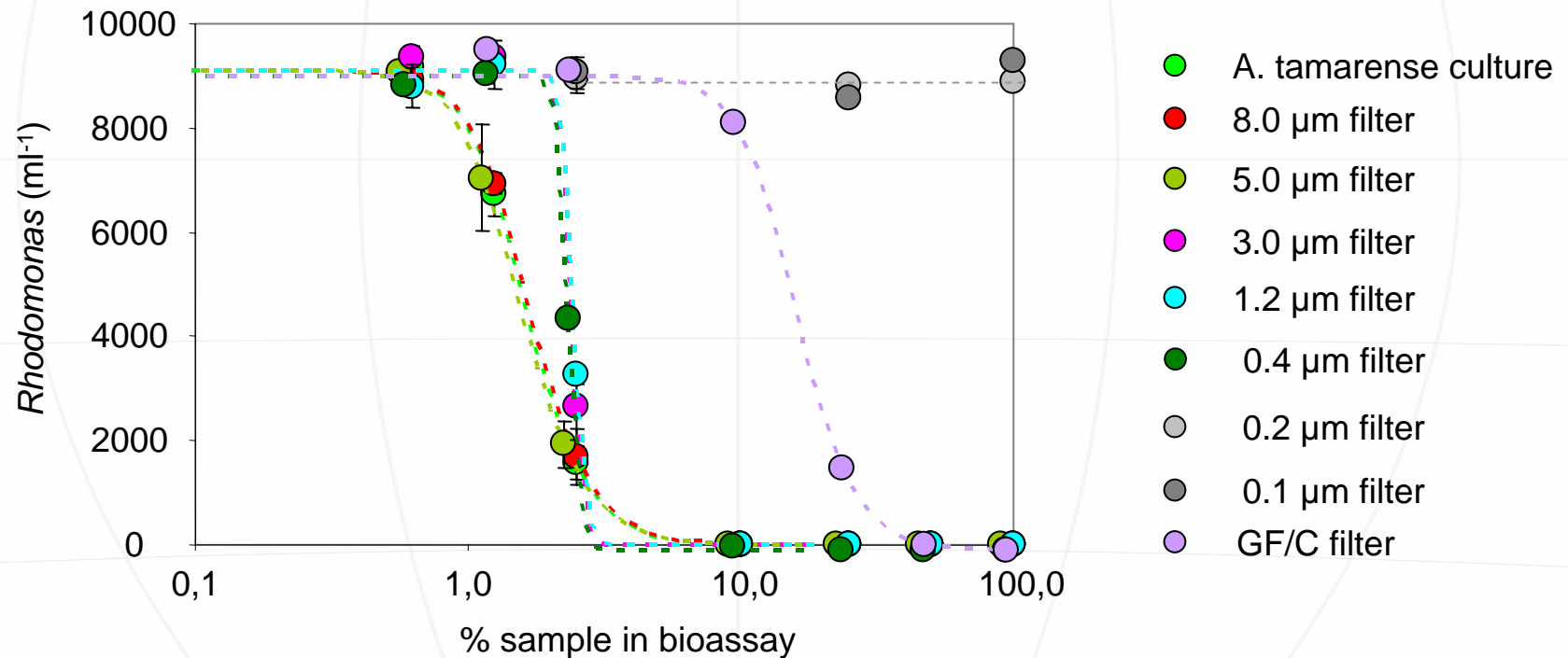
● t = 1d

● t = 7d

● t = 20d

# Chemical Experiments – Filterability

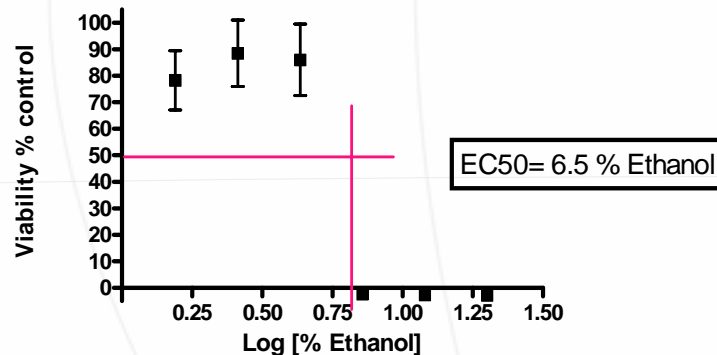
*Alexandrium tamarens* supernatant – Lytic Effect on *Rhodomonas*



# Toxicological assays

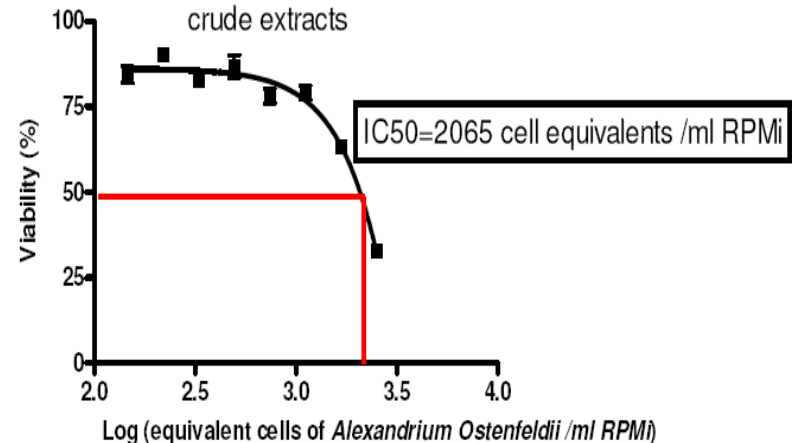
*Alexandrium ostenfeldii* extract – Toxic Effect on Neoblastoma cells

Viability curve of N2a exposed to Ethanol



Control

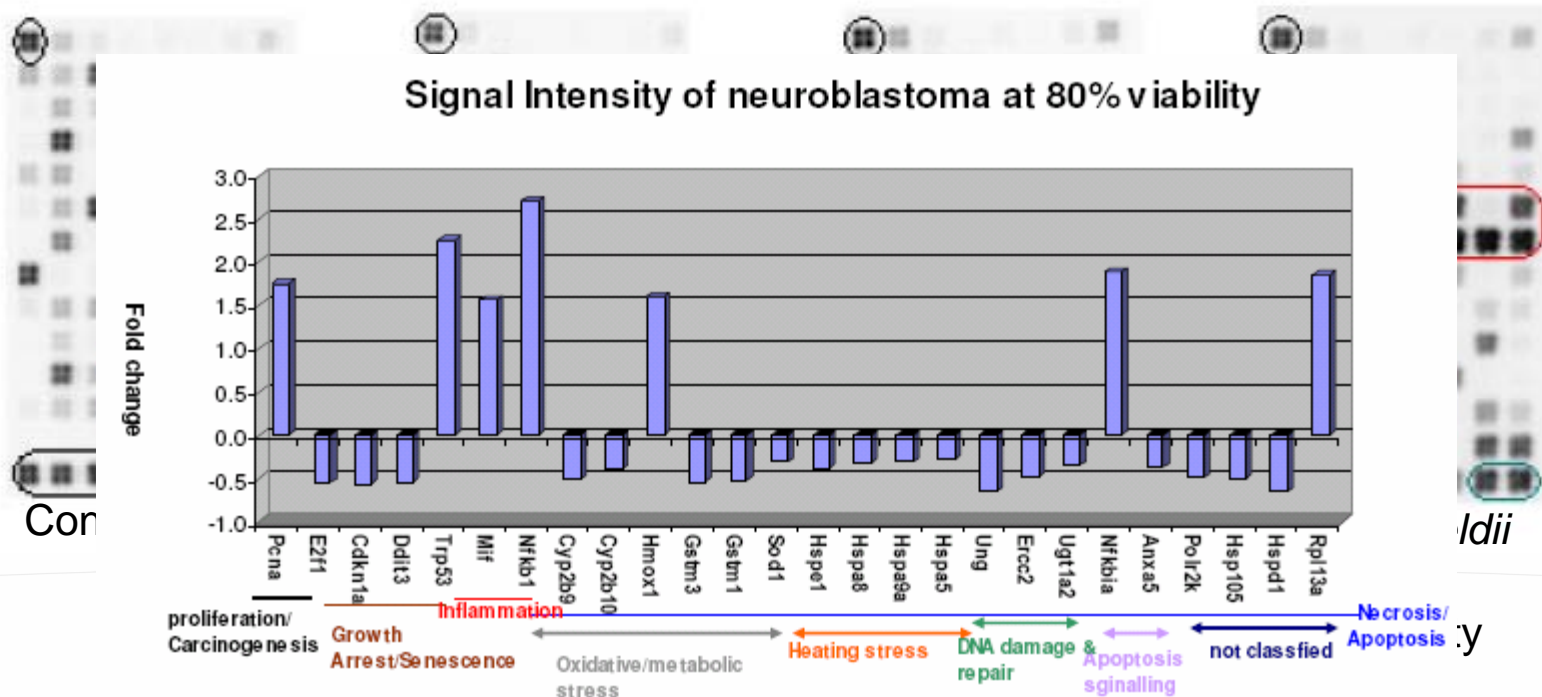
Viability curve of N2a exposed to *A. ostenfeldii* crude extracts



*A. ostenfeldii* cell extracts

# Toxicological assays

*Alexandrium ostenfeldii* extract – Expression of stress and toxicity related genes  
(GEArray Q Series Mouse Stress & Toxicity Pathway Finder)





## **AWI**

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Urban Tillmann  
Bernd Krock  
Tilman Alpermann  
Sascha Klöpfer  
Ines Jung

Sára Beszteri  
Nina Jaeckisch  
Ines Marschallek  
Chishimba M. Kantu  
Chibo Chikwililwa  
Annegret Müller  
Wolfgang Drebing

## **GKSS**

Andreas Prange  
Jürgen Gandraß  
Sandra Schäfer  
Beritt Schwalger

**Thank You**  
  
**for**  
  
**Your Attention!**